Original Article

Efficacy of Selective Phenolic Compounds on the Activity of Voltage-gated K^+ Current in Human Prostate Cancer Cell Line

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Abstract

Background and Aim: Phenolic compounds are reported to possess wide range of therapeutic properties against variety of diseases including cancer. Voltage-gated K^+ channels (I_K) are known to contribute many basic cellular functions in cancer cells. However, only few studies describe the I_K current blockade and inhibition of cancer cell growth in prostate cancer cells. To investigate the electrophysiological characteristics of I_K channels in prostate cancer cells. **Methods:** In the present study, whole-cell patch-clamp technique is used to study the modulatory effect of curcumin, rutin, troxerutin, and resveratrol on I_K current in human prostate cancer cell line PC-3. **Results:** The obtained results show that exposure of PC-3 cells to 200 μM of resveratrol inhibited I_K current more than half of the current when compared to control. However, this effect was reversible after application of external solution. Whereas curcumin, rutin, and troxerutin did not show any effect on I_K current in PC-3 cells. **Conclusion:** Our findings reveal that among the various tested compounds, only resveratrol effectively inhibited IK current in PC-3 cells and also this study concludes that not all the anticancer compounds have the ability to inhibit IK current in PC-3 cells.

Keywords: Flavonoid, I_K current, prostate cancer, stilbenes

Received: 02nd August, 2017; ***Revised:*** 18th September, 2017; ***Accepted:*** 20th September, 2017

Introduction

Prostate cancer is one of the most commonly diagnosed diseases and the sixth leading cause of cancer-related death worldwide.[1] It is diagnosed in the seventh decade of life, and hence, there have been no major advances in the treatment of diseases.[2] There are many apoptotic regulators and genetic factors involved in the onset, progression, and metastasis of human prostate cancer malignancy.[3] Several therapeutic strategies have been developed to treat prostate cancer including surgery, radiation therapy, chemotherapy, and hormonal therapy, but clinical management of metastatic prostate cancer is most challenging.[2] Therefore, there is an urgent need to develop new therapeutic targets for treating prostate cancer. In these aspects, voltage-gated K^+ channels (I_K) are new potentially important molecular therapeutic target in prostate cancer therapy.[4]

I_K channels in the plasma membranes contribute to many cellular functions including cell proliferation, volume regulation, cell migration, and cell death. Most of these functions are important for cancer cell survival and metastasis.[5] Accumulating evidence suggests that I_K channels are quite prominently expressed in human prostate cancer cells (PC-3 and LNCaP).[6] The malignant nature of these cell lines is distinguished by their ion channel characteristics. Compared with LNCaP cell lines, PC-3 cells expressed lower density of I_K current which potentially contributes to apoptotic resistance.[7] Several studies emphasized that I_K channel blockers inhibit cell proliferation in many types of cancer cells including prostate cancer.[4,8,9] Therefore, investigation on the influence of novel anticancer compounds on the activity of I_K channels seems to be putative target for prostate cancer treatment.

Phytochemicals are phenolic compounds, which are ubiquitous in vegetables and fruits. These phenolic compounds are shown to possess wide range of biological activity, including anticancer, antimetastatic, antioxidant, and anti-inflammatory

Access this article online

Quick Response Code:

Website: www.ijcep.org

DOI: 10.4103/ijcep.ijcep_39_17

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How to cite this article: George K, Malathi R. Efficacy of Selective Phenolic Compounds on the Activity of Voltage-gated K^+ Current in Human Prostate Cancer Cell Line. Int J Clin Exp Physiol 2017;4:133-8.

activities.[10,11] The anticancer properties of phenolic compounds have been shown to inhibit the initiation and progression by modulating many signaling pathways in different cancer cell types and animal models. Phenolic compounds are structurally classified into alkaloids, anthocyanins, carotenoids, coumestans, flavan-3-ols, flavonoids, hydroxycinnamic acids, isoflavones, lignans, monophenols, monoterpenes, organosulfides, phenolic acids, phytosterols, saponins, stilbenes, triterpenoids, and xanthophylls.[12] Out of these distinct classes of phytochemicals, studies have most extensively focused and reported the role of flavonoids in prostate cancer therapy. In an attempt to identify novel I_K channel modulators in PC-3 cells, we have screened a number of flavonoids (curcumin, rutin, troxerutin) and stilbenes (resveratrol).

In the present study, we aimed to investigate the electrophysiological characteristics of I_K channels in prostate cancer cells, particularly focusing on the modulatory effects of selected phenolic compounds on the I_K current in human prostate cancer cell line PC-3 using whole-cell patch-clamp technique.

Materials and Methods

Reagents

Curcumin, rutin, resveratrol, and troxerutin were purchased from Sigma (St. Louis, MO, USA). The stock solutions were prepared in DMSO and were stored at −20°C. All the drug solutions were freshly prepared from stock solutions before each set of experiments. The final concentration of DMSO was <0.1%.

Cell culture

PC-3 cells were sourced from the National Center for Cell Science (Pune, India). PC-3 cells were cultured in Ham's F12-K medium (HiMedia laboratories, India) supplemented with 10% fetal bovine serum and with 1% antibiotics (penicillin 100 IU/ml and streptomycin 100 mg/ml) in a humidified incubator at 37°C supplemented with 5% CO_2.

Electrophysiology

Whole-cell patch recordings were performed on PC-3 cells. Recordings were made at room temperature. Pipettes were pulled from borosilicate glass capillaries with resistances of 2–3 MΩ when filled with internal solution. Currents were recorded using an the Axopatch 200B (Axon Instruments, Sunnyvale, CA), Digidata 1322A (Axon Instruments), and PClamp software (version 6.0.3, Axon Instruments). The access resistance in our experiments was approximately 5–10 MΩ, and 40%–60% series resistance compensation was achieved. Current records were acquired at 5 kHz and filtered at 2 kHz. The external solution used to record K^+ currents contained the following (in mM): NaCl 140, KCl 5, $MgCl_2$ 1, D-glucose 10, and HEPES 10, adjusted to pH 7.4 with 1 M NaOH. The internal solution contained the following (in mM): KCl 140, NaCl 5, $CaCl_2$ 2, $MgCl_2$ 1, D-glucose 10, and HEPES 10, adjusted to pH 7.2 with 1 M KOH. To evaluate the effect of phenolic compounds on the I_K currents, the cells were held at a voltage of −80 mV, and membrane potential was stepped from −120 mV to +70 mV for 200 ms at 30 s intervals, respectively. All the recordings were performed with leak subtraction. The cell under investigation was continuously perfused with the external and drug solutions using the Octaflow (ALA instruments) perfusion system.

Statistical analysis

The current-voltage curves were analyzed on ClampFit (9.2.1.9), Igor Pro (5.04B), and Microsoft Excel 2012. All data values were calculated as mean ± standard error of the mean. Statistical significance of paired *t*-test and $P < 0.05$ were considered.

Results

The effects of curcumin, rutin, and troxerutin on K^+ currents in PC-3 cells

We characterized 3 flavonoids (curcumin, rutin, and troxerutin) for their modulatory activities on I_K current in human prostate cancer cell line PC-3. Depolarizing step pulse from −120 to +70 mV for 200 ms at 30 s was used to record the whole cell I_K currents in PC-3 cells [Figure 1, top left]. The representative current traces before and after the exposure of 200 μM of curcumin are shown in Figure 1a. Current-voltage (I-V) curves for I_K currents are established from the active currents [Figure 1b]. The I-V curve confirms that 200 μM of curcumin did not cause any effect on PC-3 cells.

We further externally perfused 200 μM of rutin on PC-3 cells. The representative current traces before and after the exposure of 200 μM of curcumin are shown in Figure 2a. The I-V relationship in the absence and presence of rutin are constructed [Figure 2b]. Rutin did not exert any effect on PC-3 cells. Finally, among flavonoids, we screened 200 μM of troxerutin externally. The current traces show no sign of inhibition of I_K currents in PC-3 cells [Figure 3a]. The I-V curve also confirms that there is no significant change in the presence of troxerutin at this dosage [Figure 3b] the Axopatch 200B (Axon Instruments, Sunnyvale, CA).

The effects of resveratrol on K^+ currents in PC-3 cells

Because of no sign of inhibitory effect of curcumin, rutin, and troxerutin on I_K current in PC-3 cells, we further selected resveratrol, a stilbenes, to characterize whether it exerts any inhibitory potential on Ik current in PC-3 cells inhibitory activity on I_K currents in PC-3 cells. A depolarizing step from −120 to +70 mV for 200 ms at 30 s was used to record the whole cell I_K currents in PC-3 cells [Figure 4, middle]. Superimposed current traces before and after the exposure of 200 μM of resveratrol were shown in Figure 4a. Figure 4b shows the I-V curves of I_K currents of control and 200 μM of resveratrol. The peak current density plot confirms that 200 μM resveratrol blocked I_K currents in PC-3 cells [Figure 4c]. However, this effect was reversible immediately after the exposure of washout. These results confirm that 200 μM of resveratrol blocked I_K currents in almost half of the I_K current in PC-3 cells.

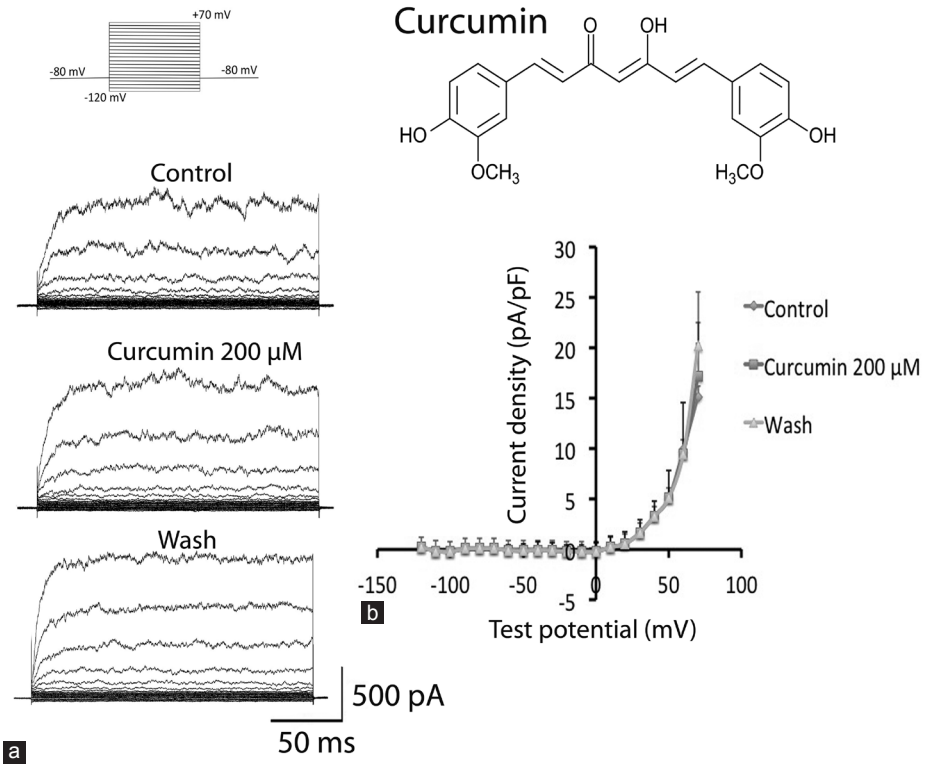

Figure 1: The effects of curcumin on I_K current in PC-3 cells. (a) Representative traces of I_K currents recorded in the presence and absence of curcumin 200 µM in PC-3 cells. (b) The current-voltage (I-V) relationships of I_K currents in the absence and presence of curcumin in PC-3 cells. Data are plotted as mean ± standard error of the mean ($n > 7$)

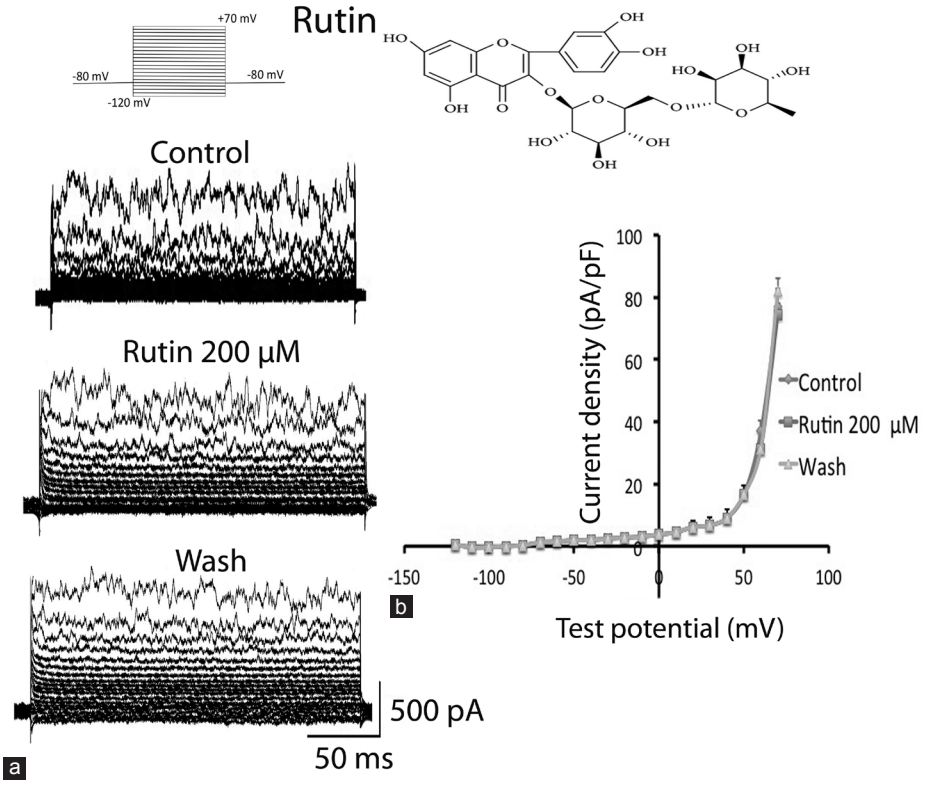

Figure 2: The effects of rutin on I_K current in PC-3 cells. (a) Representative traces of I_K currents recorded in the presence and absence of rutin 200 µM in PC-3 cells. (b) The current-voltage (I-V) relationships of I_K currents in the absence and presence of rutin in PC-3 cells. Data are plotted as mean ± standard error of the mean ($n > 7$)

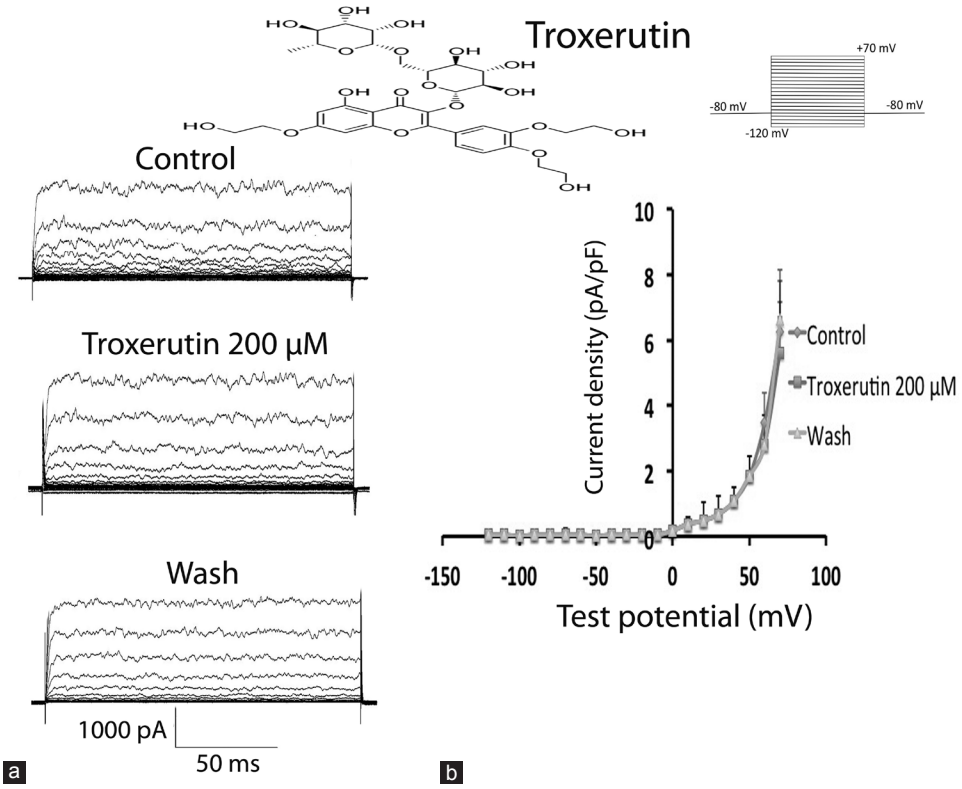

Figure 3: The effects of troxerutin on I_K current in PC-3 cells. (a) Representative traces of I_K currents recorded in the presence and absence of troxerutin 200 µM in PC-3 cells. (b) The current-voltage (I-V) relationships of I_K currents in the absence and presence of troxerutin in PC-3 cells. Data are plotted as mean ± standard error of the mean ($n > 7$)

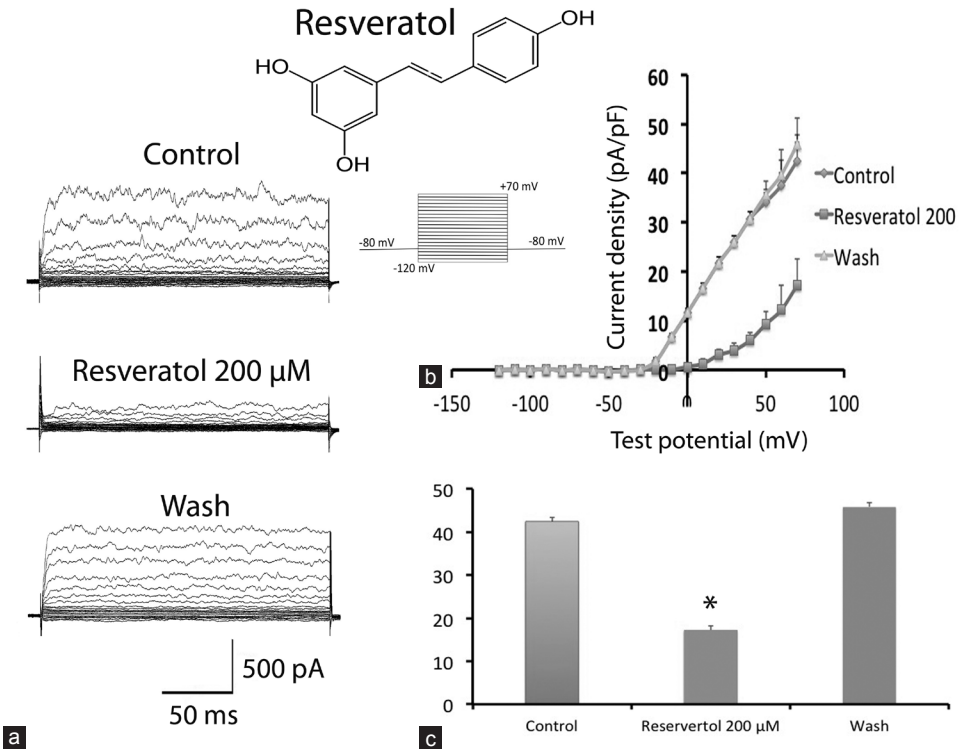

Figure 4: The effects of resveratrol on I_K current in PC-3 cells. (a) Representative traces of I_K currents recorded in the presence and absence of resveratrol 200 µM in PC-3 cells. (b) The current-voltage (I-V) relationships of I_K currents in the absence and presence of resveratrol in PC-3 cells. Data are plotted as mean ± standard error of the mean ($n > 7$). (c) Peak current densities of LNCaP cells at +70 mV in the absence and presence of resveratol. *indicates the statistically significant difference from control ($P<0.05$)

Discussion

In the present study, we have electrophysiologically characterized the effects of selected natural phenolic compounds (curcumin, rutin, troxerutin, and resveratrol) on I_K current in human prostate cancer cells. The results provide the evidence that except resveratrol, the applied concentration of 200 μM of other tested compounds appeared to be not effective inhibitors of I_K current. This study suggests that not all the anticancer compounds have the ability to inhibit I_K current in PC-3 cells.

The obtained results show that 200 μM of resveratrol is an effective inhibitor of I_K current and is also an effective anticancer compounds in PC-3 cells. However, this I_K current inhibition is reversible after perfusion of external solution. In addition, resveratrol has shown to exhibit activation of autophagic cell death in PC-3 cells through modulating several signaling pathways.[13] Besides, 200 μM of resveratrol has shown inhibition of voltage-gated K^+ channel $K_V1.3$ in human lymphocytes. Furthermore, resveratrol accounted concentration- and time-dependent inhibition of $K_V1.3$ current.[14] Likewise, resveratrol has shown to inhibit both delayed rectifier K^+ current (I_K) and fast transient K^+ (I_A) currents in rat hippocampal neurons.[15] However, our obtained results show that 200 μM of resveratrol inhibited more than half of the I_K current in PC-3 cells.

Flavonoids are the most effective class of phenolic compounds with common structure of two aromatic rings connected to three carbon atoms. Our obtained data indicate that flavonoids such as curcumin, rutin, and troxerutin exerted no inhibitory effect on I_K current in PC-3 cells. However, these compounds have shown to exert anticancer and antineoplastic effect in many studies, targeting multiple signaling pathways.[16,17] For example, curcumin is known as an effective anticancer compound for many cancers including prostate cancer.[18] However, curcumin also has shown to inhibit several types of voltage-gated K^+ channel in various cancer cells. Curcumin reversibly inhibited Kv 1.4 K^+ current in adrenal zona fasciculata cells,[19] Kv2.1 current in human embryonic kidney 293 cells,[20] human ether-a-go-go-related gene in acute monocytic leukemia cell line (THP-1),[21] and voltage-dependent K^+ channels in rabbit coronary arterial smooth muscle cells.[22] However, our obtained results clearly show that 200 μM of curcumin does not cause any effect on I_K current in PC-3 cells.

Our results show that resveratrol, which has less hydroxyl groups in the molecular structure as compared to flavonoids, effectively inhibited I_K current in PC-3 cells. It was also reported as effective inhibitors of prostate cancer cell growth and proliferation. However, among several classifications of phenolic compounds, flavonoids exerted several physiological activities including anticancer activities, but our results show no marked inhibition of I_K current in PC-3 cells at 200 μM of concentration. There is no correlation of I_K current inhibition and anticancer properties of curcumin, rutin, and troxerutin. At present, thus, we cannot ignore the hypothesis that these tested compounds are ineffective on I_K current in PC-3 cells because they might inhibit the I_K current in PC-3 cells at higher concentrations. Thus, it can be suggested that the I_K channel inhibition depends on the affinity of channel protein with the molecular structure of the compounds.

Limitations of the study

The concentration of the compounds used in the present study may not exactly represent the concentration required *in vivo*.

Conclusion

Our findings reveal that among the various tested compounds, only resveratrol effectively inhibited IK current in PC-3 cells and also this study concludes that not all the anticancer compounds have the ability to inhibit IK current in PC-3 cells.

Acknowledgment

We gratefully acknowledge the funding support from University grant commission, New Delhi, for providing grant in the form of Basic Scientific Research fellowship for meritorious students (JRF) (No. F.7-376/2012 [BSR]).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Jain S, Saxena S, Kumar A. Epidemiology of prostate cancer in India. Meta Gene 2014;2:596-605.
2. Bommareddy A, Eggleston W, Prelewicz S, Antal A, Witczak Z, McCune DF, *et al.* Chemoprevention of prostate cancer by major dietary phytochemicals. Anticancer Res 2013;33:4163-74.
3. Singh SK, Banerjee S, Acosta EP, Lillard JW, Singh R. Resveratrol induces cell cycle arrest and apoptosis with docetaxel in prostate cancer cells via a p53/p21WAF1/CIP1 and p27KIP1 pathway. Oncotarget 2017;8:17216-28.
4. Fraser SP, Grimes JA, Djamgoz MB. Effects of voltage-gated ion channel modulators on rat prostatic cancer cell proliferation: Comparison of strongly and weakly metastatic cell lines. Prostate 2000;44:61-76.
5. Lang F, Stournaras C. Ion channels in cancer: Future perspectives and clinical potential. Philos Trans R Soc Lond B Biol Sci 2014;369:20130108.
6. Laniado ME, Fraser SP, Djamgoz MB. Voltage-gated K(+) channel activity in human prostate cancer cell lines of markedly different metastatic potential: Distinguishing characteristics of PC-3 and LNCaP cells. Prostate 2001;46:262-74.
7. Prevarskaya N, Skryma R, Bidaux G, Flourakis M, Shuba Y. Ion channels in death and differentiation of prostate cancer cells. Cell Death Differ 2007;14:1295-304.
8. Skryma RN, Prevarskaya NB, Dufy-Barbe L, Odessa MF, Audin J, Dufy B, *et al.* Potassium conductance in the androgen-sensitive prostate cancer cell line, LNCaP: Involvement in cell proliferation. Prostate 1997;33:112-22.
9. Fieber LA, González DM, Wallace MR, Muir D. Delayed rectifier K currents in NF1 schwann cells. Pharmacological block inhibits proliferation. Neurobiol Dis 2003;13:136-46.
10. Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. Nutr J 2016;15:99.
11. Haddad AQ, Venkateswaran V, Viswanathan L, Teahan SJ, Fleshner NE, Klotz LH, *et al.* Novel antiproliferative flavonoids induce cell cycle

arrest in human prostate cancer cell lines. Prostate Cancer Prostatic Dis 2006;9:68-76.
12. Singh AN, Baruah MM, Sharma N. Structure based docking studies towards exploring potential anti-androgen activity of selected phytochemicals against prostate cancer. Sci Rep 2017;7:1955.
13. Selvaraj S, Sun Y, Sukumaran P, Singh BB. Resveratrol activates autophagic cell death in prostate cancer cells via downregulation of STIM1 and the mTOR pathway. Mol Carcinog 2016;55:818-31.
14. Teisseyre A, Michalak K. Inhibition of the activity of human lymphocyte kv1.3 potassium channels by resveratrol. J Membr Biol 2006;214:123-9.
15. Gao ZB, Hu GY. Trans-resveratrol, a red wine ingredient, inhibits voltage-activated potassium currents in rat hippocampal neurons. Brain Res 2005;1056:68-75.
16. Thomas NS, George K, Arivalagan S, Mani V, Siddique AI, Namasivayam N, *et al.* The *in vivo* antineoplastic and therapeutic efficacy of troxerutin on rat preneoplastic liver: Biochemical, histological and cellular aspects. Eur J Nutr 2017;56:2353-66.
17. Wei X, Zhou D, Wang H, Ding N, Cui XX, Wang H, *et al.* Effects of pyridine analogs of curcumin on growth, apoptosis and NF-κB activity in prostate cancer PC-3 cells. Anticancer Res 2013;33:1343-50.
18. Chen M, Zhou B, Zhong P, Rajamanickam V, Dai X, Karvannan K, *et al.* Increased intracellular reactive oxygen species mediates the anti-cancer effects of WZ35 via activating mitochondrial apoptosis pathway in prostate cancer cells. Prostate 2017;77:489-504.
19. Liu H, Danthi SJ, Enyeart JJ. Curcumin potently blocks Kv1.4 potassium channels. Biochem Biophys Res Commun 2006;344:1161-5.
20. Aréchiga-Figueroa IA, Delgado-Ramírez M, Morán-Zendejas R, Rodríguez-Menchaca AA. Modulation of Kv2.1 channels inactivation by curcumin. Pharmacol Rep 2015;67:1273-9.
21. Banderali U, Belke D, Singh A, Jayanthan A, Giles WR, Narendran A, *et al.* Curcumin blocks Kv11.1 (erg) potassium current and slows proliferation in the infant acute monocytic leukemia cell line THP-1. Cell Physiol Biochem 2011;28:1169-80.
22. Hong DH, Choi IW, Son YK, Kim DJ, Na SH, Jung WK, *et al.* The effect of PI3 kinase inhibitor LY294002 on voltage-dependent K(+) channels in rabbit coronary arterial smooth muscle cells. Life Sci 2013;92:916-22.